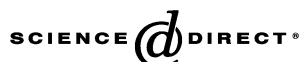


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Modification of myosin protein and gene expression in failing hearts due to myocardial infarction by enalapril or losartan

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Abstract

The effects of enalapril, an angiotensin converting enzyme (ACE) inhibitor, and losartan, an angiotensin II receptor type I antagonist, were investigated on alterations in myofibrillar ATPase activity as well as myosin heavy chain (MHC) content and gene expression in failing hearts following myocardial infarction (MI). Three weeks after ligation of the left coronary artery, rats were treated with or without enalapril (10 mg/kg/day), and/or losartan (20 mg/kg/day) for 5 weeks. The infarcted animals exhibited an increase in left ventricle (LV) end diastolic pressure and depressed rates of LV pressure development as well as pressure decay. LV myofibrillar Ca^{2+} -stimulated ATPase activity was decreased in the infarcted hearts compared with controls, MHC α -isoform content was significantly decreased whereas that of MHC β -isoform was markedly increased. The level of MHC α -isoform mRNA was decreased whereas that of MHC β -isoform was increased in the viable infarcted LV. Treatment of animal with enalapril, losartan, or combination of enalapril and losartan partially prevented the MI induced changes in LV function, myofibrillar Ca^{2+} -stimulated ATPase activity, MHC protein expression and MHC gene expression. The results suggest that the beneficial effects of the renin–angiotensin system blockade in heart failure are associated with partial prevention of myofibrillar remodeling. © 2004 Elsevier B.V. All rights reserved.

Keywords: Myosin heavy chain isoform; Myocardial infarction; Congestive heart failure; Myofibrillar ATPase; Cardiac gene expression; Angiotensin converting enzyme inhibitor; Angiotensin II receptor antagonist

1. Introduction

The ability of the cardiac muscle to generate contractile force is primarily dependent upon myofibrillar Ca^{2+} -stimulated ATPase activity [1]. Furthermore, it is known that the myofibrillar ATPase activity is determined by different amounts of myosin heavy chain (MHC) isozymes, namely α -MHC and β -MHC in the myocardium [2,3]. A shift in the composition of myosin isozymes with respect to α -MHC and β -MHC contents has been shown to depress myosin ATPase as well as myofibrillar Ca^{2+} -stimulated ATPase activities and contractile function in different models of cardiac hypertrophy and heart failure [3–8]. Recently, we have observed that a significant shift from α -MHC to β -MHC was associated with depressed myosin ATPase and myofibrillar

Ca^{2+} -stimulated ATPase activities in failing hearts due to myocardial infarction (MI) in rats [9]. However, the mechanisms of such a change in the molecular composition of myofibrils (myofibrillar remodeling) in congestive heart failure (CHF) due to MI are not understood. Since the renin–angiotensin system (RAS) is activated in CHF and its blockade has been shown to prevent cardiac remodeling (changes in cardiomyocyte size and shape) and improve heart function in CHF due to MI in humans [10,11] and animal models [9,12–15], it is likely that myofibrillar remodeling in the failing heart is prevented by the blockade of RAS. This view is based on our observation that treatment of infarcted rats with imidapril, an angiotensin converting enzyme (ACE) inhibitor, was found to attenuate depressions in left ventricular (LV) function and myofibrillar Ca^{2+} -stimulated ATPase activity as well as the changes in MHC protein and gene expression [9]. No information is available at present whether the effect of imidapril on myofibrillar remodeling in the failing heart is simulated by other ACE inhibitors or angiotensin II receptor antagonists.

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Although enalapril, a widely used ACE inhibitor, and losartan, an angiotensin II receptor (AT₁R) antagonist, have been reported to produce beneficial actions on cardiac remodeling and heart failure [15–17], the effects of enalapril and losartan on changes in myofibrillar Ca²⁺-stimulated ATPase and MHC isoforms in CHF remain to be examined. Thus, the present study was undertaken to investigate if improvement of cardiac function is associated with prevention of changes in myosin isozymes and gene expression for α -MHC and β -MHC, as well as myofibrillar Ca²⁺-stimulated ATPase activities in the failing heart upon treatment with enalapril or losartan. In order to test if the effects of enalapril and losartan are additive, infarcted animals were treated with a combination of both drugs.

2. Materials and methods

2.1. Experimental model

MI was induced in male Sprague–Dawley rats (175–200 g) by occlusion of the left coronary artery as described earlier [18–20]. Briefly, the heart in anesthetized animals was exposed through left thoracotomy and the left coronary artery was ligated at about 2 mm from origin of the aorta. The heart was repositioned in the chest and the incision was closed with a purse string suture. Sham-operated rats were treated in the same way except that the artery was not ligated. Mortality of experimental rats was approximately 35% within 48 h. Electrocardiography was performed before and after open-chest to test the success of operation, and also performed at 3 weeks following MI. All experimental protocols were approved by the Animal Care Committee of the University of Manitoba following guidelines established by the Canadian Institutes of Health Research.

2.2. Treatment with enalapril and/or losartan

All animals received standard care, kept at 12-h day/night cycle, fed regular rat chow and were provided water ad libitum. The experimental animals were randomly divided into five groups in this project: sham-operated (Sham), infarcted (MI), enalapril treated infarcted (ENP), losartan treated infarcted (LOS), and combined enalapril and losartan treated infarcted (COM). Three weeks after the operation, enalapril (10 mg/kg/day) and/or losartan (20 mg/kg/day), or tap water was given orally via a gastric tube to sham and infarcted groups for 5 weeks. The selection of the doses for these drugs was based on our previous study showing the beneficial effects of these agents on the sarcoplasmic reticulum protein and gene expression in this experimental model [15]. Enalapril and losartan were supplied by Merck Research Laboratories (Rahway, NJ, USA).

2.3. Hemodynamic studies

At 8 weeks post-surgery, the animals were anesthetized with an intraperitoneal injection of a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg) and the right carotid artery was exposed. A cannula with a microtip pressure transducer (model SPR-249, Millar Instruments, Houston, TX, USA) was introduced through proximal arteriotomy [18,19]. The readings of pressures in aorta and LV were taken from a computer program (AcqKnowledge for Windows 3.0, Harvard Apparatus, Montreal, Canada). After the hemodynamic measurement of LV end-diastolic pressure (LVEDP), rate of pressure development (+dP/dt), and rate of pressure decay (–dP/dt) in the anesthetized animals, the hearts were quickly removed, the LV (including septum) and the scar tissue were dissected, weighed, frozen in liquid nitrogen and stored at –70 °C. The lung wet/dry wt ratio, an index of pulmonary congestion, as well as the heart wt/body wt ratio, an index of cardiac hypertrophy, were measured in these animals.

Since scar wt/total LV wt (including septum and infarcted tissue) ratio was found to exhibit a linear relationship with infarct size (as measured morphometrically) [9,15], the scar wt was used as a marker to determine the extent of scar size. It should be pointed out that about 10% of untreated and treated animals showed small infarct (scar wt/total LV ratio <15% corresponding to scar size <30% of the free LV wall). Thus, the hemodynamic data from the animals showing small infarct were not included and the cardiac tissue from these animals was discarded.

2.4. Myofibrillar Mg²⁺-stimulated ATPase and Ca²⁺-stimulated ATPase activities

Myofibrils were isolated according to the procedure employed earlier [9,21] and suspended in a final solution containing 100 mM KCl, 20 mM Tris–HCl (pH 7.0). Mg²⁺-stimulated ATPase activity was determined at 30 °C in a medium containing 20 mM imidazole (pH 7.0), 2 mM MgCl₂, 2 mM Na₂ATP, 10 mM NaN₃, 1.6 mM ethylenegluco-bis (β -aminoethylether) *N,N,N',N'*-tetraacetic acid (EGTA) and 50 mM KCl. Total ATPase activity was determined in the same medium except that EGTA was replaced by 1 μ M of free of Ca²⁺. All reactions were terminated after 5 min by the addition of 1 ml of 12% trichloroacetic acid and the samples were centrifuged; phosphate in the protein-free supernatant was determined [22]. Ca²⁺-stimulated ATPase activity was taken as the difference between values obtained for total and Mg²⁺-stimulated ATPase activities.

2.5. Relative protein quantification of cardiac myosin

Relative protein contents of cardiac MHC and myosin light chain (MLC) were determined by Western blot [9].

The muscle was homogenized using a Brinkmann homogenizer with Kinematica 87/Polytron PTA 7K1 for 4–6 s in homogenizing buffer (100 μ l buffer per 2 mg of muscle), containing 60 mM KCl, 1 mM cysteine, 20 mM imidazole (pH 6.9), 1 mM $MgCl_2$, 1 mM ouabain, 10 mM NaN_3 , 1 mM $CaCl_2$, 0.01% leupeptin, 250 M phenylmethylsulfonylfluoride and 1 mM dithiothreitol (DTT). The concentration of protein in the homogenate was adjusted to 1 mg/ml with the homogenizing buffer. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer [0.25 M Tris-HCl (pH 6.8), 8% (w/v) sodium dodecyl sulfate, 45% glycerol, 20% β -mercaptoethanol, and 0.006% bromophenol blue] was added into the homogenate buffer (1:3) [23]. The samples were boiled for 5 min at 95 °C. The proteins in homogenate separated by SDS-PAGE were electroblotted to Immobilon-P transfer membrane (Millipore Company, Bellerica, MA, USA) in a transfer buffer [25 mM Tris-HCl, 120 mM glycine and 20% methanol (v/v)]. The transferred membranes were shaken for 2 h in blocking buffer, which contained TBS (10 mM Tris-HCl, 150 mM NaCl) solution and 5% fat-free powdered milk, then incubated for 1 or 2 h at room temperature with monoclonal anti-MHC antibody, anti-MLC mouse IgG antibody, or anti-TnI antibody (1:1000, Sigma Immuno Chemicals, St. Louis, MO, USA). The transferred membranes were subsequently incubated with biotinylated anti-mouse IgG (1:1000, Amersham Biosciences, Baie d'Urfe, Canada) and then streptavidin conjugated horseradish peroxidase (1:5000, Amersham). The blots were rinsed in the TBS-T (10 mM Tris-HCl, 150 mM NaCl and 0.2% Tween-20) solution several times. For chemiluminescent detection, the membrane sheets were developed on Hyperfilm-ECL (Amersham) to visualize proteins. The relative protein content was determined by a GS-670 Imaging Densitometer (Bio-Rad Company, Hercules, CA, USA) with the Image Analysis Software Version 1.0.

2.6. Analysis of cardiac MHC isoforms

Cardiac MHC isoforms were determined under denaturing conditions. The α -MHC and β -MHC isoforms in the

SDS-PAGE samples were separated by 4% SDS-PAGE as described [5,9,24]. Electrophoresis was carried out at a constant 220 V for 3–3.5 h with cooling between 13 and 17 °C and continuous stirring of bottom tank buffer. The gels were stained with Coomassie brilliant blue R250 and destained with 7% acetic acid by diffusion. Relative amounts of isoforms were estimated by Imaging Densitometer (GS-670, Bio-Rad).

2.7. RNA isolation and Northern blot analysis

Total myocardial RNA preparation was extracted from the viable LV of sham, MI, ENP, LOS, COM rats by the acid guanidinium thiocyanate–phenol–chloroform method (TRIzol reagent, GIBCO-BRL Life Technologies, Paisley, UK) according to the manufacturer's instructions. The RNA concentration was calculated from the absorbance at 260 and 280 nm. Steady-state levels of α -MHC and β -MHC mRNA were determined by Northern hybridization analysis [9]. After transferring, covalently cross-linked to the matrix using UV radiation (UV Stratalinker 2400, Stratagene, Cedar Creek, TX, USA), each membrane was hybridized with ^{32}P -labeled cDNA probes at 42 °C. Cardiac α -MHC, β -MHC, MLC, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probes (American Type Culture Collection) were labelled by random primer DNA labelling system using Klenow fragment. Filters were exposed to X-ray film (Kodak X-OMAT) at –80 °C with intensifying screens. Results of autoradiographs from Northern blot analysis were quantified by densitometry (Bio-Rad imaging densitometer GS 670). The signal of α -MHC, β -MHC and MLC mRNA was normalized to that of GAPDH mRNA to account for differences in loading and/or transfer.

2.8. Data analysis

Data are expressed as mean \pm S.E.. The differences among various groups were evaluated statistically by one-way ANOVA followed by the Newman–Keuls test. A P value <0.05 was taken to represent a significant difference.

Table 1

General characteristics of myocardial infarcted rats with or without enalapril, and/or losartan treatment for 5 weeks starting at 3 weeks after coronary occlusion

	Sham	MI	ENP	LOS	COM
Body weight (g)	505 \pm 11	503 \pm 13	499 \pm 19	505 \pm 22	500 \pm 23
Heart weight (mg)	1210 \pm 90	1610 \pm 110*	1421 \pm 68 [#]	1387 \pm 28 [#]	1341 \pm 27 [#]
Scar wt (mg)	ND	230 \pm 15	231 \pm 18	228 \pm 11	229 \pm 16
Heart wt/BW (mg/g)	2.40 \pm 0.12	3.20 \pm 0.25*	2.85 \pm 0.22 [#]	2.74 \pm 0.13 [#]	2.68 \pm 0.20 [#]
Lung wet/dry wt	4.35 \pm 0.1	5.51 \pm 0.33*	4.85 \pm 0.18 [#]	4.90 \pm 0.30 [#]	4.95 \pm 0.31 [#]
Liver wet/dry wt	3.16 \pm 0.3	3.26 \pm 0.22	3.21 \pm 0.16	3.19 \pm 0.20	3.34 \pm 0.16

Values are mean \pm S.E. of seven animals in each group. ENP: myocardial infarcted treated with enalapril; LOS: myocardial infarcted treated with losartan; MI: myocardial infarcted; COM: myocardial infarcted treated with both enalapril and losartan; ND: not detected.

* $P < 0.05$ compared with Sham group.

[#] $P < 0.05$ compared with MI group.

3. Results

3.1. General characteristics and hemodynamic changes

Occlusion of the left coronary artery resulted in scar formation in the LV, while the remaining cardiac muscle in the 8-week infarcted animals underwent hypertrophy as indicated by increased ventricular wt compared to the sham control value (Table 1). A significant increase in wet wt/dry wt ratio of the lungs indicated the presence of pulmonary congestion in MI rats. The liver wet wt/dry wt ratio was not altered. The depression in contractile function in the 8-week untreated MI group was evident from an increase in LVEDP and a decrease in both $+dP/dt$ and $-dP/dt$ (Table 2). Changes in these parameters for general characteristics and hemodynamics were partially (significantly but not completely) prevented by treatment with enalapril, losartan or combination of enalapril and losartan. Furthermore, MAP in the untreated and treated groups was not different from that in the sham control animals (Table 2). The alterations in treated and untreated MI groups observed in this study are similar to those reported earlier [9,13,15]. It was interesting that the combination therapy did not produce additive effects as these values were not different from those obtained by treatments with either enalapril or losartan alone (Tables 1 and 2). The scar wt in the untreated MI group was not different from the treated groups.

3.2. Myofibrillar ATPase activities

Myofibrils isolated from the viable LV of infarcted hearts exhibited a lower ($7.1 \pm 0.4 \mu\text{mol Pi/mg/h}$) Ca^{2+} -stimulated ATPase activity ($P < 0.05$) compared with sham-operated rats ($10.8 \pm 0.4 \mu\text{mol Pi/mg/h}$). Blockade of RAS with enalapril and/or losartan partially normalized ($P < 0.05$) the activity (8.3 ± 0.65 , 7.9 ± 0.52 , and $8.4 \pm 0.58 \mu\text{mol Pi/mg/h}$) in ENP, LOS and COM groups, respectively (Fig. 1). The values for Ca^{2+} -stimulated ATPase activity in COM group were not different from that in ENP or LOS groups. Furthermore, no alterations of

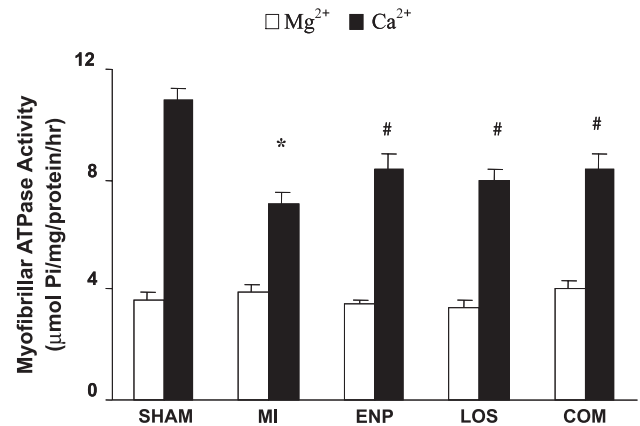


Fig. 1. Myofibrillar Mg^{2+} - and Ca^{2+} -stimulated ATPase activities of the left ventricles from sham, infarcted (MI), enalapril-treated infarcted (ENP), losartan-treated infarcted (LOS), and combined enalapril and losartan-treated infarcted (COM) rats. ENP and/or LOS were given orally for 5 weeks starting at 3 weeks after inducing MI. Values are means \pm S.E. of seven animals for each group. * $P < 0.05$ vs. Sham; # $P < 0.05$ vs. MI.

myofibrillar Mg^{2+} -stimulated ATPase activity were found among different groups (Fig. 1).

3.3. Protein content of MHC isoforms

The relative protein content of MHC was determined from immunoblots using a monoclonal anti-MHC (a mixture of α -MHC and β -MHC) antibody. No significant alterations in protein contents for MHC, MLC and troponin (TnI) were observed in rats with MI with or without any drug treatments (Fig. 2). It should be noted that during polyacrylamide gel electrophoresis, β -MHC exhibited a higher electrophoretic mobility than α -MHC, which was the dominant (>90%) isoform in sham group (Fig. 3). In MI group, protein content for α -MHC was reduced significantly while that for β -MHC was increased markedly (Fig. 3); β -MHC content was increased from 6.5% to 29% of total MHC in the viable tissue of LV whereas α -MHC content was decreased from 93.5% to 71.0% of total MHC. Blockade of RAS with ENP, or LOS, partially prevented the increase in β -MHC as well as the decrease in α -MHC due to

Table 2

Hemodynamic parameters in myocardial infarcted rats with or without enalapril, and/or losartan treatment for 5 weeks starting at 3 weeks after coronary occlusion

	Sham	MI	ENP	LOS	COM
Heart rate (bpm)	265 \pm 9	270 \pm 13	272 \pm 19	258 \pm 15	267 \pm 15
LVSP (mm Hg)	122 \pm 6.5	118 \pm 4.6	115 \pm 8.5	116 \pm 9.5	114 \pm 8.6
LVEDP (mm Hg)	4.2 \pm 0.3	18.9 \pm 4.6*	8.9 \pm 1.2#	9.0 \pm 0.7#	8.5 \pm 0.6#
+dP/dt (mm Hg/s)	11,228 \pm 850	8409 \pm 687*	9521 \pm 459#	9362 \pm 562#	9533 \pm 625#
−dP/dt (mm Hg/s)	12,518 \pm 1050	8524 \pm 950*	10,057 \pm 1012#	9895 \pm 762#	9959 \pm 721#
MAP (mm Hg)	102 \pm 9	100 \pm 10	95 \pm 7	101 \pm 7	97 \pm 5

Values are mean \pm S.E. of seven animals in each group. ENP: myocardial infarcted treated with enalapril; LOS: myocardial infarcted treated with losartan; COM: myocardial infarcted treated with both enalapril and losartan; LVEDP: left ventricular end diastolic pressure; LVSP: left ventricular systolic pressure; MAP: mean arterial pressure; MI: myocardial infarcted.

* $P < 0.05$ compared with Sham group.

$P < 0.05$ compared with MI group.

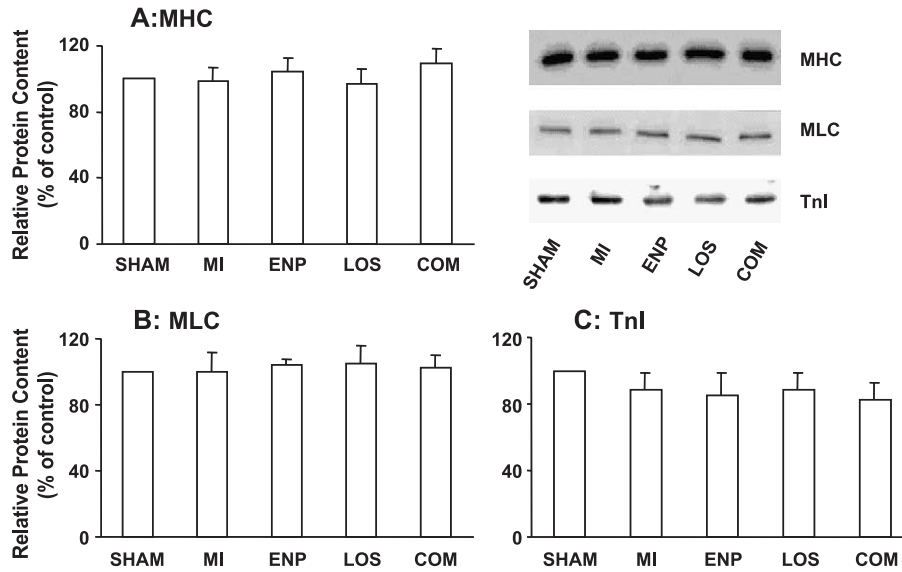


Fig. 2. Typical immunoblots and relative protein contents of myosin heavy chain (MHC) (A), myosin light chain (MLC) (B), and troponin I (TnI) (C) in the left ventricles from Sham, infarcted (MI), enalapril-treated infarcted (ENP), losartan-treated infarcted (LOS), and combined enalapril and losartan-treated infarcted (COM) rats. ENP and/or LOS were given orally for 5 weeks starting at 3 weeks after inducing MI. Values are means \pm S.E. of seven animals for each group. * $P < 0.05$ vs. Sham; # $P < 0.05$ vs. MI.

MI. Furthermore, the combination therapy did not show any additive effects (Fig 3).

3.4. Gene expression for α -MHC and β -MHC

The mRNA levels for α -MHC and β -MHC were determined by Northern blot analysis (Fig. 4). The α -MHC mRNA level was decreased by 39% and that for β -MHC mRNA was increased by 125% in LV from the infarcted

rats. These changes were partially reversed by treatment with ENP, LOS and COM. The mRNA levels for MLC in the sham group were not different from those in the untreated and drug treated MI groups (Fig. 4).

4. Discussion

In this study, heart failure due to MI was found to be associated with depressed myofibrillar Ca^{2+} -stimulated ATPase activity and a shift in MHC isozymes. Both $+dP/dt$ and $-dP/dt$ were decreased whereas LVEDP was markedly increased indicating cardiac dysfunction in the infarcted animals. In addition, a decrease in myofibrillar ATPase activity, an increase in β -MHC protein and gene expression, as well as a decrease in α -MHC protein and gene expression were also evident in hearts following MI. These hearts were hypertrophied as the heart wt/body wt ratio was increased and the animals showed signs of CHF as the lung wet wt/dry wt ratio was also increased. These results, showing depressed cardiac function and myofibrillar ATPase activity as well as changes in MHC protein and gene expression, are in agreement with our results reported previously in this experimental model [9,25–27]. In view of the critical role of α -MHC and β -MHC in determining the velocity of cardiac contraction [1–4], it is likely that the depressed myofibrillar Ca^{2+} -stimulated ATPase activity in the failing heart may be due to the observed increase in β -MHC protein content and a decrease in α -MHC protein content. Since the protein content of total MHC was not altered, it appears that there occurs a shift in MHC isozymes in the failing myocardium. Such a change in the protein contents of MHC isozymes seems specific because the protein content

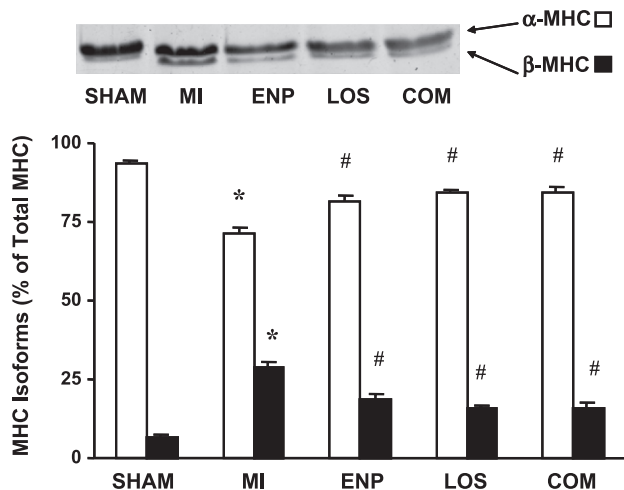


Fig. 3. Myosin heavy chain α - and β - isozymes (α -MHC and β -MHC) in left ventricles from Sham, infarcted (MI), enalapril-treated infarcted (ENP), losartan-treated infarcted (LOS), and combined enalapril and losartan-treated infarcted (COM) rats. The figure shows typical immunoblots for α -MHC and β -MHC-isozymes and results for laser densitometric analysis. ENP and/or LOS were given orally for 5 weeks starting at 3 weeks after inducing MI. Values are means \pm S.E. of seven animals for each group. * $P < 0.05$ vs. Sham; # $P < 0.05$ vs. MI.

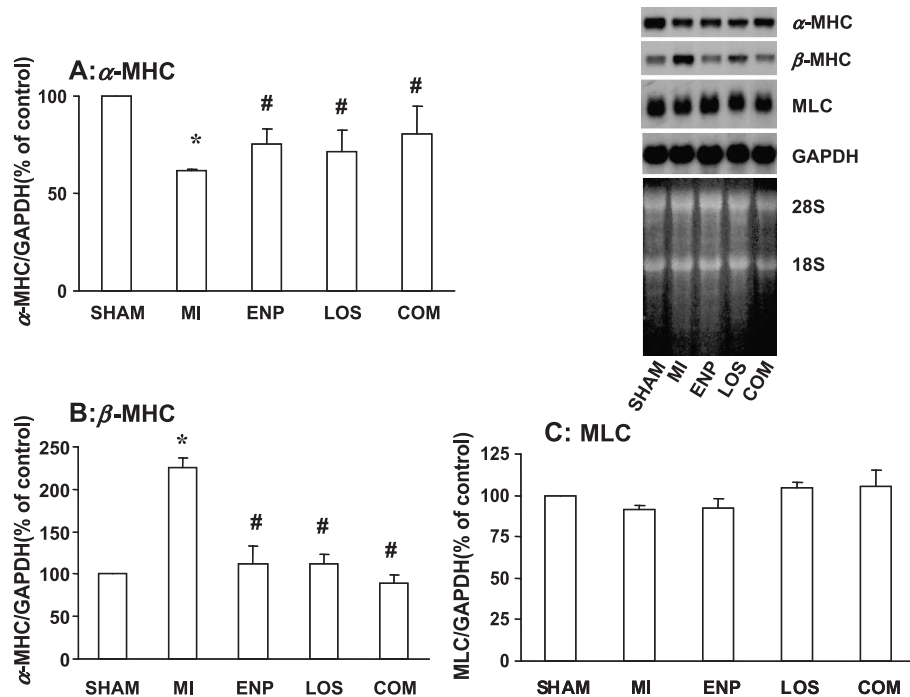


Fig. 4. Typical Northern blots showing mRNA abundance for myosin heavy chain α -isozyme (A), myosin heavy chain β -isozyme (B), and myosin light chain (C) in left ventricles from Sham, infarcted (MI), enalapril-treated infarcted (ENP), losartan-treated infarcted (LOS), and combined enalapril and losartan-treated infarcted (COM) rats. α -MHC: mRNA blot for myosin heavy chain α -isozyme; β -MHC: mRNA blot for myosin heavy chain β -isozyme; MLC: mRNA blot for myosin light chain; GAPDH: mRNA blot for glyceraldehyde-3-phosphate dehydrogenase; 28S/18S: the quality of mRNA preparation is evident from the ethidium bromide staining of the 28S and 18S ribosomal RNA. Values are means \pm S.E. of seven animals for each group. * P < 0.05 vs. Sham; # P < 0.05 vs. MI.

of both MLC and TnI in the failing hearts was unchanged. Since we did not determine the absolute values for the MHC isoforms in the failing heart by employing mass spectroscopy [28], some caution should be exercised while interpreting the observed changes in α -MHC and β -MHC in terms of quantitative alterations in myofibrillar protein contents. Nonetheless, the observed alterations in the relative values for protein contents of MHC isoforms can be seen to result in changes in the composition as well as molecular structure of myofibrils and thereby represent the process of myofibrillar remodeling during the development of heart failure. This process of myofibrillar remodeling in the failing heart may occur at the level of gene expression as mRNA levels for α -MHC were decreased and those for β -MHC were increased. Since such a remodeling has also been shown to occur in the sarcoplasmic reticulum in CHF due to MI [15], it is likely that remodeling of both sarcoplasmic reticulum and myofibrils may be involved in the development of cardiac dysfunction in the failing heart.

Treatment of infarcted animals with enalapril, an ACE inhibitor, was observed to partially prevent alterations in cardiac hypertrophy, lung congestion, heart function, myofibrillar ATPase activity and a shift in MHC isoform protein and gene expression. Since neither enalapril nor losartan affected the protein contents and mRNA levels for MLC, it is evident that the observed effect of these drugs in MHC protein and gene expression is specific in nature. These results are consistent with the beneficial effects of imidapril,

an ACE inhibitor, reported earlier in this experimental model [9]. Since the effect of both enalapril and imidapril were simulated by losartan, an AT_1R antagonist, it appears that the beneficial actions of both enalapril and imidapril on heart function and cardiac as well as myofibrillar remodeling are due to the blockade of RAS in animals with heart failure. Both enalapril and losartan as well as other ACE inhibitors such as captopril and trandolapril have also been shown to partially prevent cardiac remodeling and changes in sarcoplasmic reticular function, protein content and gene expression in the failing hearts [13,15,29,30]. Furthermore, treatment of infarcted animals with imidapril was found to partially prevent changes in sarcolemmal phospholipase C isoform expression [31]. Thus, it is evident that the blockade of RAS may play a critical role in preventing cardiac and subcellular remodeling. However, it is unlikely that the beneficial effects of the RAS blockade on cardiac and subcellular remodeling in infarcted animals are due to reduction in the afterload because we did not observe any change in the MAP upon treatments with ACE inhibitors or losartan. Since ACE inhibitors are also known to prevent the breakdown of bradykinin [32,33], it can be argued that the beneficial effects of both enalapril and imidapril are mediated through the actions of bradykinin. Although we have not carried out any experiment to rule out this possibility, this mechanism in the experimental model used may not be of any major significance. This view is based on our observation that the combination therapy with both enalapril

and losartan did not produce an additive effect on heart dysfunction or myofibrillar remodeling.

5. Conclusions

The results presented here demonstrate that the depressed myofibrillar ATPase activity and the shift in α -MHC and β -MHC protein and gene expressions due to MI were partially prevented by enalapril or losartan. These observations lend further support to the concept regarding the occurrence of subcellular remodeling in heart failure [34]. Since the combined therapy with enalapril and losartan prevents changes in heart function and myofibrillar remodeling partially, it appears that other mechanisms such as the activation of sympathetic nervous system [32,35,36], in addition to the activation of RAS, may also be participating in the genesis of cardiac dysfunction and myofibrillar remodeling during the development of heart failure. Thus, extensive work needs to be carried out to understand the exact mechanisms for the development of cardiac dysfunction and subcellular remodeling in different experimental models of CHF.

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